Pergamon

Acta Zoologica (Stockholm), Vol. 75, No. 3, pp. 255–267, 1994
Elsevier Science Ltd

0001–7272/94$7.00+ .00

Relationship of Homolidae and Dromiidae: Evidence from Spermatozoal Ultrastructure (Crustacea, Decapoda)

D. Guinot,* B. G. M. Jamieson† and B. Richer de Forges‡

*Laboratoire de Zoologie (Arthropodes), Muséum national d'Histoire naturelle, 61 rue Buffon, F-75231 Paris Cedex 05, France
†Zoology Department, University of Queensland, Brisbane 4072, Queensland, Australia
‡ORSTOM, B.P. A5, Nouméa Cedex, Nouvelle-Calédonie

(Accepted for publication 15 June 1993)

Abstract

Guinot, D., Jamieson, B. G. M. & Richer de Forges, B. 1994. Relationship of Homolidae and Dromiidae: evidence from spermatozoal ultrastructure (Crustacea, Decapoda).—*Acta Zoologica* (Stockholm) 75: 255–267.

The homolid spermatozoon, as exemplified by *Homola* sp., *Paromola* sp. and *Paromola petterdi*, differs markedly from spermatozoa of crabs of the Heterotremata–Thoracotremata assemblage but agrees with the sperm of dromiids, in the strongly anteroposteriorly depressed acrosome (apomorphy?) and the capitate form of the perforatorium (a major synapomorphy seen nowhere else in the Crustacea). These similarities support inclusion of the Dromiidae and Homolidae in a single grouping, the Podotremata. The homolid perforatorium differs from that of dromiids in the autapomorphic spiked-wheel form of the anterior expansion. Homolid spermatozoa show nuclear arms symplesiomorphic of all investigated crabs (small or questionably sometimes absent in Dromiidae), and corresponding loss of purely microtubular arms seen in other reptants. Homolid sperm agree with those of dromiids (synapomorphy?), raninids, higher heterotremes and thoracotremes (homoplasies?) but differ from lower heterotremes, in lacking microtubules in the nuclear arms. A posterior median process of the nucleus in homolids, not seen in dromiids, is shared with anomurans and lower heterotremes. No features in the ultrastructure of homolid or dromiid sperm have been detected which associate them exclusively with either the Raninidae or the heterotreme and thoracotreme Brachyura.

B. G. M. Jamieson, Zoology Department, University of Queensland, Brisbane 4072, Queensland, Australia.

Introduction

Guinot (1977, 1978, 1979, 1991) divides the Brachyura (crabs) into three sections mainly on the basis of the location of the male and female pores: the Podotremata (containing the Dromiacea), the Heterotremata and the Thoracotremata. The coxal positions of male and female pores and isolation of the spermathecae from the oviducts, with external fertilization, characterizing the podotremes, were considered by Guinot (1978, p. 218) to be an ensemble of plesiomorphies (symplesiomorphies). Jamieson (1990, p. 126) considered that this symplesiomorphic definition left the validity of the Podotremata in some doubt. The Podotremata diagnosed by Guinot (1978, 1979) contain not only the Dromioidea and Homolodromioidea (both comprising the restricted subsection Dromiacea) but also the Homoloidea, Raninoidea, and Cyclodorippoidea (formerly Tymoloidea), all three comprising a subsection Archaeobrachyura which Guinot, at that time, admitted was a grade. The superfamily Homoloidea de Haan, 1839, which included three families (Homolidae de Haan, 1839; Latreilliidae Stimpson, 1858; Poupiniidae Guinot, 1991) had long been associated with the Dromiacea and many workers subordinated the Homoloidea in the Dromiacea. The Heterotremata and Thoracotremata share a synapomorphy in the sternal location of the female pores and development of the spermatheca as a sternal vulva on sternite 6 allowing for internal fertilization. The Thoracotremata are further apomorphic in the constant sternal location of the male pores.

In transferring the Homoloidea to the Archaeobrachyura, Guinot (1979) listed morphological characters of the adult, notably the absence of uropods, features of the thoracic sternum and the axial thoracic skeleton, which separated the Homoloidea from the Dromiacea. Based on larval morphology, Williamson (1965, 1974) and Rice (1980, 1981*a*,*b*) excluded the Dromioidea from the Brachyura while the Homoloidea were retained. However, a polyphyletic origin of the Brachyura was found unacceptable to Balss (1957) and to paleontologists (e.g. Glaessner 1969; Wright & Collins 1972) who retain the Dromiacea in the Brachyura.

With regard to wider dromiacean (*sensu lato*) and brachyuran relationships, it has been debated whether dromiaceans arose at the base of all crabs, from within the macrurans, or from basal anomurans. Glaessner (1969) considered there to be good palaeontological evidence that the Dromiacea (*sensu lato*, including homolids) arose from within the Glypheoidea, a macruran group related to spiny lobsters (Palinura). It has been acknowledged that specialized features of the zoea larvae of the Dromiidae are not brachyuran and the larvae have been considered distinctly like those of anomurans, having a

shrimp-like shape, persistent uropods, and functional third maxillipeds (Warner 1977; Williamson 1974). More precisely, the Dromiacea have been attributed an origin near, or from, the Thalassinidea, and therefore at a level more primitive than most Anomura (*sensu strictu*) (Burkenroad 1963; Gurney 1942; Pike & Williamson 1960; Rice 1980, 1983; Williamson 1965, 1974; see also discussions in Stevcic 1971; Guinot 1979). Both Dromiacea (specifically dynomenids) and Thalassinidea first appear in the fossil record at the beginning of the Jurassic (Glaessner 1969). A large number of fossils attributable to the Homolidae are known since the mid-Jurassic with a conspicuous radiation in the Cretaceous; the known fossil genera virtually disappeared in the Tertiary. The appearance of homolids is thus earlier than the Cretaceous origin (e.g. Dorippidae) of heterotrematous and thoracotrematous brachyurans.

As to relationships of homolids and raninids, the interpretation of homolid relationships from larval morphology and ontogeny has been somewhat equivocal (Williamson 1988) but has tended to endorse an origin of homolids near the base of the Heterotremata–Thoracotremata–raninid assemblage. Thus, on the basis of ontogenetic criteria, Williamson (1965, 1974) recognizes profound differences considered to separate Homolidae and Dromiacea and corresponding with a very ancient bifurcation: homolid larvae are primitive and at a level equivalent to that of anomuran larvae but have particular characters suggesting that they represent a pre-brachyuran stock; the Dromiidae can be excluded from the Brachyura. Rice (1970, 1980) and Rice & Provenzano (1970) expressed similar views: homolid and raninid larvae present similarities which suggest that they belong to a pre-brachyuran stock. It was concluded (Rice 1981*a*,*b*, 1983) that the Dromioidea were close to the Anomura, that homolids arose near the base of the higher Brachyura but that apomorphic characters shared by the zoeae of raninids and higher brachyura, but not by homolids, indicate that homolids became separated from a primitive brachyuran line at an earlier stage than the raninids. For Rice (1980, p. 298, fig. 9) 'the modern larval condition in the homolids, raninids and the higher Brachyura have all evolved from a more primitive homolid which possessed larval characters common to all three'. Finally, Williamson (1988, 1992) explained the dromiacean paradox by invoking horizontal gene transfer, giving anomuran larvae but brachyuran adults.

Nucleotide sequences of 18S ribosomal RNA support the exclusion of the Dromiidae from the Brachyura and inclusion of the Raninidae in the Brachyura (Spears & Abele 1988; Abele 1991; Spears *et al.* 1993). In the latter work the Dromiidae appear paraphyletic; *Hypoconcha* is the sister-taxon of the Anomura (*Clibanarius*) while *Dromia* is at the base of the raninid–heterotreme assemblage.

In the present study we describe the spermatozoal ultrastructure of three homolid species, collected off New Caledonia, in an attempt to elucidate homolid relationships: *Homola* sp. and *Paromola* sp., and a new genus to receive *Paromola* (formerly *Latreillopsis*) *petterdi* (Grant 1905), all three taxa described by Guinot and Richer de Forges (in press). Sperm of a dromiid, a raninid and a heterotreme are illustrated for comparative purposes.

Materials and Methods

Specimens of the three homolid species were collected by B. Richer de Forges during the BERYX 11 Cruise on the R.V. 'Alis' (13–23 October 1992), South of New Caledonia on the guyots of the Norfolk Ridge. Portions of the testes and male ducts were fixed in 3% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2), with 3% sucrose, at 4°C for 2 h and despatched in the fixative to Brisbane for further processing. On receipt in Brisbane they were washed in buffer; post-fixed for 80 min in similarly buffered 1% osmium tetroxide; washed in three 15 min changes of buffer; dehydrated through an ethanol series; and infiltrated and embedded in Spurr's epoxy resin. Sections were cut with diamond knives, on an LKB 2128 UM IV microtome. Thin sections, 50–80 nm thick, were collected on carbon stabilized colloidin-coated 200 mesh copper grids, stained for 30 s in lead citrate, rinsed in distilled water, stained for 1 min in 6% aqueous uranyl acetate, rinsed in distilled water, stained for a further 30 s in lead citrate, before final rinsing. Electron micrographs were taken on an Hitachi 300 electron microscope at 75 kV and a JEOL 100 at 60 kV.

Specimens of *Ranina ranina* (Linné, 1758) were collected from Heron Island, Great Barrier Reef, in October 1984 and of *Portunus pelagicus* (Linné, 1758) from Moreton Bay, in February 1988. Specimens of *Petalomera lateralis* (Gray, 1831) were collected from One Tree Island, Great Barrier Reef, in December 1988, all localities in Queensland, Australia (for ultrastructural procedures see Jamieson 1989*a* and 1990, respectively).

Results

General

A generalized homolid sperm is illustrated semidiagrammatically in Fig. 1. The bulk of the homolid spermatozoon consists of an ellipsoidal acrosome bordered posteriorly by the irregular nucleus. A thick zone of cytoplasm, containing degenerating mitochondria and tortuous membranes intervenes between the acrosome and nucleus. The longitudinal axis of the spermatozoon is occupied by a wide cylindrical, anteriorly widening column, identified as a perforatorium, which is capitate anteriorly by virtue of lateral expansion near its tip. The expansion does not form a continuous flange but is subdivided into laterally directed horizontal spikes, radiating in the form of a spiked-wheel, or the ribs of an umbrella, and contained within the anterior material of the acrosome. A low dome-shaped dense layer, with a wide apical interruption, covers the anterior limit of the perforatorium and its spikes and extends laterally over much of the anterior aspect of the acrosome vesicle; this layer is identifiable with the operculum of the sperm of anomurans, dromiids, raninids and higher crabs. It is covered by the general acrosome membrane and the plasma membrane of the sperm cell.

Acrosome

The acrosome is a thick disc, domed centrally at its free, polar surface (Figs 1, 2A, 3A, B, 4A, B, 5A). Dimensions of the acrosome, width and anteroposterior length are, respectively: 3.96–4.92 μm and 2.09–2.69 μm, ratio width : length 1.93, mean of 7 (*Homola* sp.); 3.79–4.67 μm and 1.85–2.31 μm, ratio width : length 2.03, mean of 3 (*Paromola* sp.); 3.46–3.68 μm and 1.97–2.09 μm, ratio width : length 1.78, mean of 3

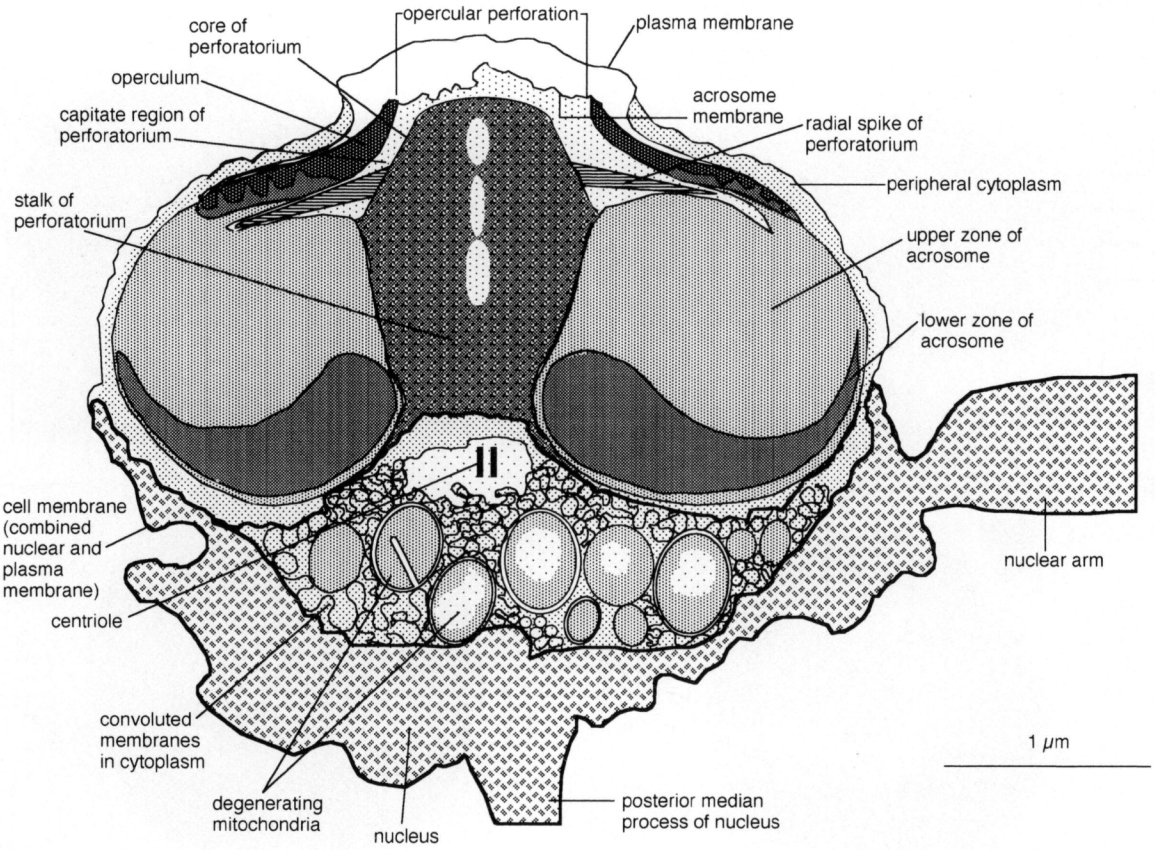

Fig. 1. Generalized homolid sperm, based on *Homola* sp., *Paromola* sp. and *Paromola petterdi* (semidiagrammatic).

(*Paromola petterdi*). This albeit small sample indicates that the acrosome is less depressed in *Paromola petterdi* than in the other two species.

The acrosome vesicle (Fig. 2A, *Homola* sp.; Figs 3A, B, *Paromola* sp.; Figs 4A, B, *Paromola petterdi*; Fig. 5A, *Homola* sp.) is bounded by a generally thin acrosomal membrane which is most clearly distinguished as a crenulate dense membrane anterior to the tip of the perforatorium. The operculum appears to be continuous with, or is at least closely contiguous with, this anterior region of the membrane, which it circumscribes. A thin moderately pale layer underlies the membrane where it bounds the acrosome vesicle, extends from the posterior limit of the operculum, around the sides and posterior face of the acrosome vesicle and is invaginated posteriorly along the posterolateral walls of the perforatorium. This pale layer may be equivalent to the capsule observed in the acrosomes of other crab sperm.

The bulk of the contents of the acrosome vesicle form an inflated ring surrounding the axial perforatorial chamber. The substance of the ring (Fig. 2A, *Homola* sp.; Figs 3A, B, *Paromola* sp.; Figs 4A, B, *Paromola petterdi*; Fig. 5A, *Homola* sp.) is subdivided into an upper, large moderately electron-dense zone, constituting most of its thickness, and a lower strongly electron dense zone which in vertical section is approximately crescent shaped with the concavity anterior. These zones are seen in transverse section in Figs 2D–F (*Homola* sp.); Figs 3D, E (*Paromola* sp.); and Fig. 4C (*Paromola petterdi*).

The upper, paler zone is directly overlain by the spikes of the perforatorium or, between these, by material resembling it in density. It is uncertain whether this overlying material is part of the upper acrosomal zone or is to be regarded as a separate subopercular zone or is, indeed, part of the operculum. Above the level of the spikes this zone is covered by and seems continuous with the dense material of the operculum. In all three species the operculum extends dense extensions into the substance of the perforatorium in the vicinity of the base of the spikes (Fig. 2A, B, *Homola* sp.). These extensions are numerous and are arranged radially.

The centre of the acrosome vesicle is penetrated by a stout vertical column of dense material which widens subapically in a capitate configuration, as seen in vertical section, composed of the radiating spines (Fig. 2A, *Homola* sp.; Figs 3A, B, *Paromola* sp.; Figs 4A, B, *Paromola petterdi*; Fig. 5A, *Homola* sp.), the whole constituting the putative perforatorium. Its stalk is circular in cross-section (Figs 2D, E, *Homola* sp.; Fig. 3D, *Paromola* sp.; Fig. 4C, *Paromola petterdi*). The transverse head of this capitate structure and the surrounding operculum occupy and account for the dome-shaped summit of the acrosome. The dense material of which the stalk and head of the perforatorium is composed is not homogeneous though forming a continuum, in *Homola* sp. (Figs 2A, 5A) and *Paromola petterdi* (Figs 4A, B); a central anteriorly tapering core, filling the entire perforatorial chamber in its posterior half, is moderately electron dense whereas the base of the spike is electron pale. In *Paromola* sp. the entire perforatorium, stalk and spikes, is moderately dense, though some suggestion of a central core may be visible (Figs 3A, B).

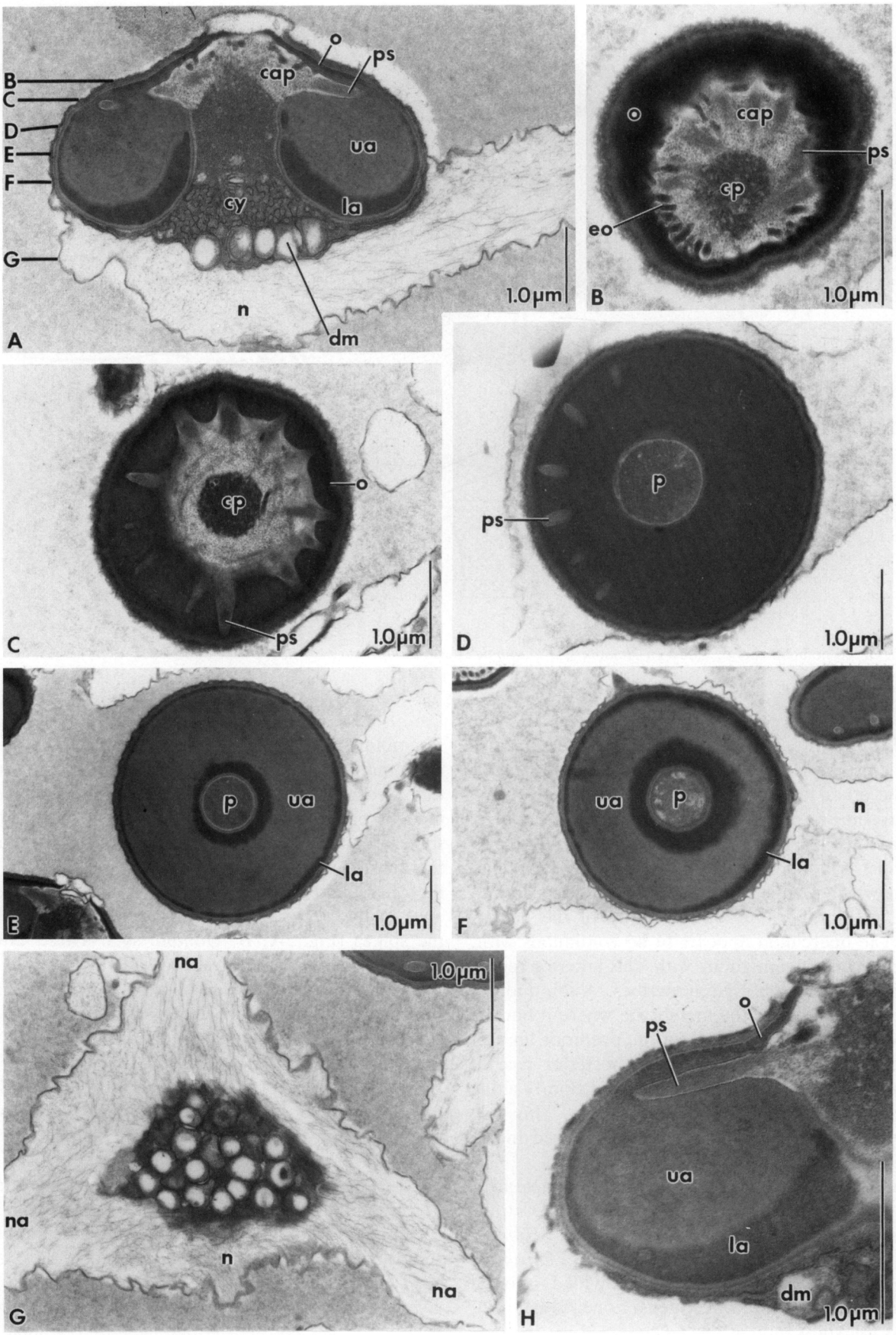

Fig. 2. Transmission electron micrographs of spermatozoa of *Homola* sp.—*A*. Sagittal longitudinal section slightly to one side of the apical hiatus in the operculum. Capital letters indicate planes of sectioning in subsequent illustrations bearing those letters.—*B*. Oblique transverse section (TS) through the operculum and supporting rays of radial spikes of perforatorium.—*C*, *D*. TS acrosome showing radial spikes of perforatorium.—*E*. TS acrosome through base of perforatorium.—*F*. TS acrosome through anterior extension of cytoplasm.—*G*. TS nucleus, at junction with cytoplasm, showing triradiate form.—*H*. Detail of LS acrosome showing perforatorial spike.

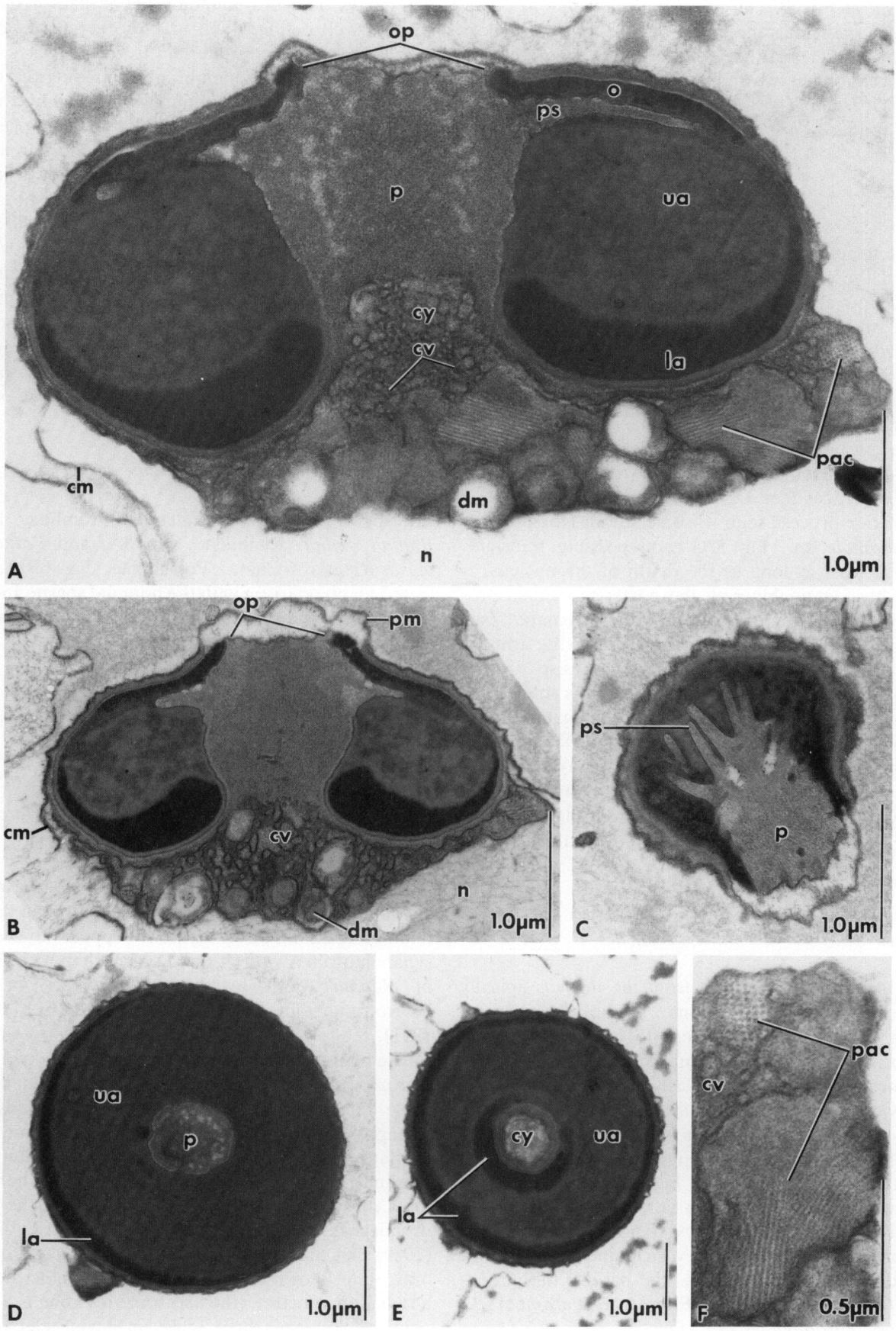

Fig. 3. Transmission electron micrographs of spermatozoa of *Paromola* sp.—*A*, *B*. Sagittal longitudinal sections.—*C*. Very oblique section showing radial spikes of head of perforatorium.—*D*. TS acrosome through base of perforatorium.—*E*. Through anterior extension of cytoplasm.—*F*. Detail of paracrystalline material in transformed mitochondria.

The radial spikes are supported by fibrous cores, radiating from the central core of the perforarium, shown for *Homola* sp. in Fig. 2C, in which there appears to be 12 spikes. The radial arrangement, as seen in transverse sections of the head of the perforatorium, is not entirely regular, occasionally two spikes arise from the same base (Figs 2B–D, *Homola* sp.; Fig. 3C, oblique, *Paromola* sp.; Fig. 4C, oblique, *Paromola petterdi*).

Nucleus

The nucleus posteriorly cups the acrosome–cytoplasm portion of the sperm (Figs 1, 2A, 3A, B, 4A, B, 5A). Its thickness is about one-third to one-half of that of the acrosome and it extends anteriorly as far as the equator of the acrosome. It is very irregular in form, sending out a few large processes laterally and posteriorly or these may not be apparent in a given longitudinal section. However, a cross-section of the nucleus at its junction with the postacrosomal cytoplasmic region (Fig. 2G, *Homola* sp.), shows the nucleus as a triradiate structure, with three vertices (see Discussion).

A posterior process seen in some sperm (for instance one of *Homola* sp., Fig. 5A), and possibly transient, which is at least as long as the depth of the nucleus, is questionably identifiable with the posterior median process of the Anomura–lower Heterotremata (see Discussion). No microtubules are present in the arms or elsewhere in the sperm. The chromatin consists of fine, diffusely arranged putative DNA fibrils but is so electron pale as scarcely to be visible. The nuclear material is in direct contact with the plasma membrane (the combined membrane being termed the cell membrane) and a discrete nuclear membrane is not visible. Anteriorly, the concavity of the nucleus is separated from the acrosome and, medianly, from the cytoplasm by a thick dense irregular membrane.

Cytoplasm centrioles and other organelles

The cell membrane continues from the nucleus apically over the surface of the acrosome, as the plasma membrane, to which it is closely adherent. No cytoplasm intervenes between the plasma membrane and the acrosome but at the anterior pole the plasma membrane is more or less widely separated from the acrosome membrane. There is some evidence that this apical separation is artefactual (Figs 2A, *Homola* sp.; Figs 3A, B, *Paromola* sp.; Figs 4A, B, *Paromola petterdi*; Fig. 5A, *Homola* sp.).

The large mass of cytoplasm lies in the hiatus at the hind end of the perforatorium, extends thinly along the posterior face of the acrosome vesicle and anteriorly for a short distance axially as far as the base of the perforatorium (Fig. 2A, *Homola* sp.; Figs 3A, B, *Paromola* sp.; Figs 4A, B, *Paromola petterdi; Fig. 5A, Homola* sp.; see also transverse sections, Figs 2F, 3E). It contains posteriorly situated subspherical bodies with dense bounding membranes, some of which have what appear to be vestigial cristae (e.g. *Paromola petterdi*, Figs 4A, B) and are therefore deduced to be degenerate mitochondria. In *Paromola* sp. (Figs 3A, F), the contents of the putative mitochondria in some sperm is replaced with paracrystalline arrays, and the bounding membrane of the bodies are less well defined than in the other two species but the paracrystalline material is not always evident (Fig. 3B). The dense membranes bounding the degenerate mitochondria are continuous with highly convoluted membranes which fill the bulk of the cytoplasm. The cytoplasm is separated from the perforatorium and acrosome by a similar dense membrane which is itself frequently infolded as part of the tortuous membranes and of those limiting the putative mitochondria. The irregular membranes bounding the posterior and anterior faces of the cytoplasm pass laterally to join, and apparently combine with, the cell membrane at the anterior limit of the nuclear cup, shortly behind the equator of the acrosome. Centrioles are probably normally present in the cytoplasm as one has been seen in an area of the cytoplasm devoid of convoluted membranes (Fig. 4B, *Paromola petterdi*).

Dromiid, raninid, and heterotreme sperm

The sperm of *Petalomera lateralis* (Dromiidae, Fig. 5B), *Ranina ranina* (Raninidae, Fig. 6A) and *Portunus pelagicus* (Heterotremata, Portunidae, Fig. 6B) are illustrated for comparison with the homolid sperm. In Fig. 5B, for *P. lateralis* an apical interruption, of the operculum, previously unrecognized, is shown.

Discussion

Comparison of homolid sperm with those of dromiids, Ranina, and heterotreme Brachyura

The spermatozoa of *Homola* sp., *Paromola petterdi* and *Paramola* sp. are very similar and constitute a distinctive homolid sperm. This nevertheless appears to share more synapomorphies with dromiid sperm than with the sperm of *Ranina* (both in the Podotremata) or of the heterotreme–thoracotreme assemblage (see Jamieson 1991).

The homolid spermatozoon differs markedly from spermatozoa of the Heterotemata–Thoracotremata assemblage but agrees with the sperm of dromiids, in the strongly anteroposteriorly depressed acrosome and the capitate form of the perforatorium. The capitate perforatorium is a major synapomorphy seen nowhere else in the Crustacea. It is noteworthy, in view of the origin of dromiaceans near the palinurids suggested by Glaessner (1969) that the acrosome of *Scyllarus chacei* provides the only other known case in Crustacea of an acrosome with a radiating structure (the acrosome ray zone is a different phenomenon on a finer scale) in having electron-dense rays (40 in number compared with only about 12 in *Homola* sp.) radiating from a dense disc which lies at the apex of the bell-shaped vesicle, under the plasma membrane, like the struts of an umbrella (McKnight & Hinsch 1986). The palinurid structure occurs in the

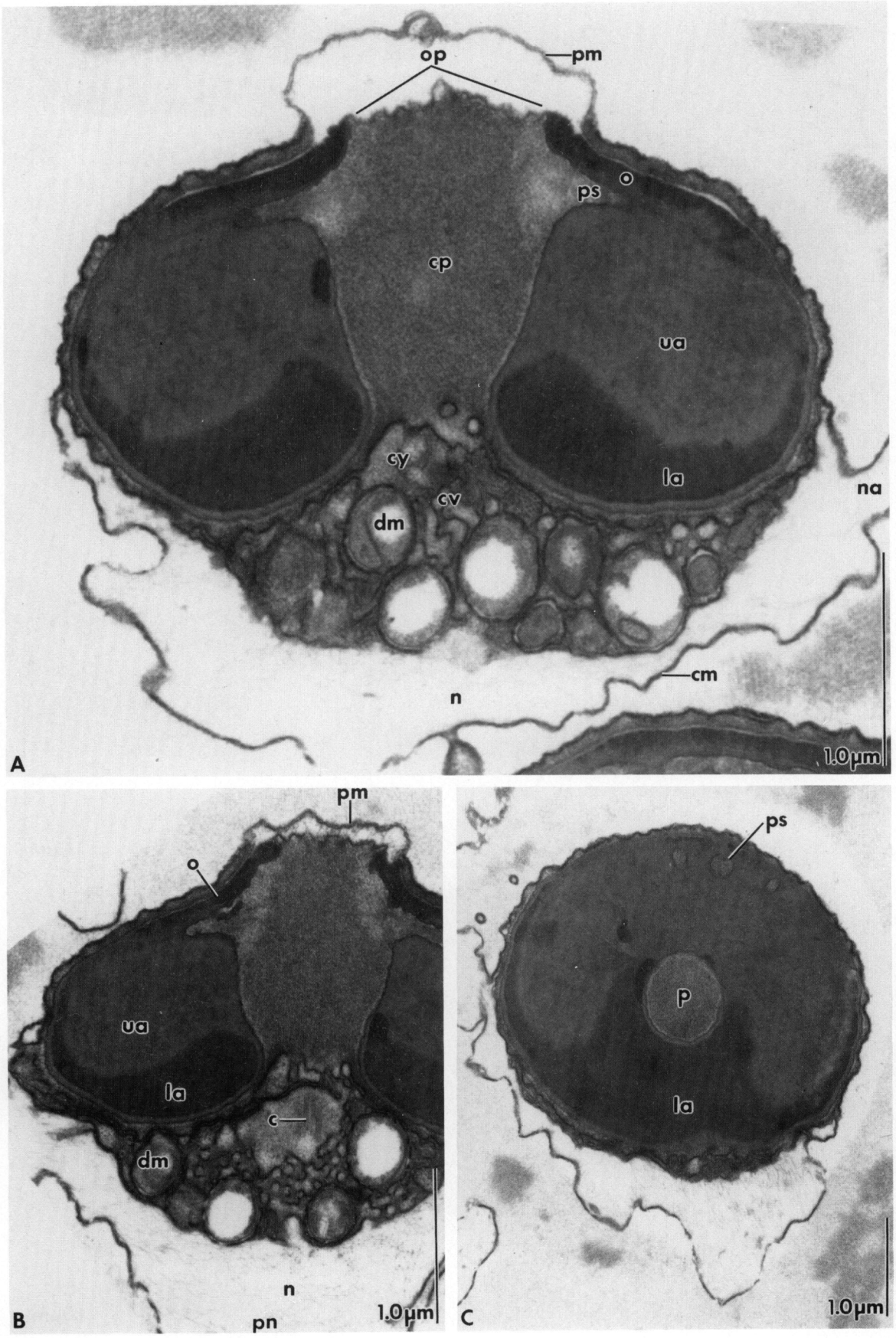

Fig. 4. Transmission electron micrographs of spermatozoa of *Paromola petterdi*.—*A*, *B*. Sagittal longitudinal sections.—*C*. Oblique TS through stalk of perforatorium and some of the radial spikes.

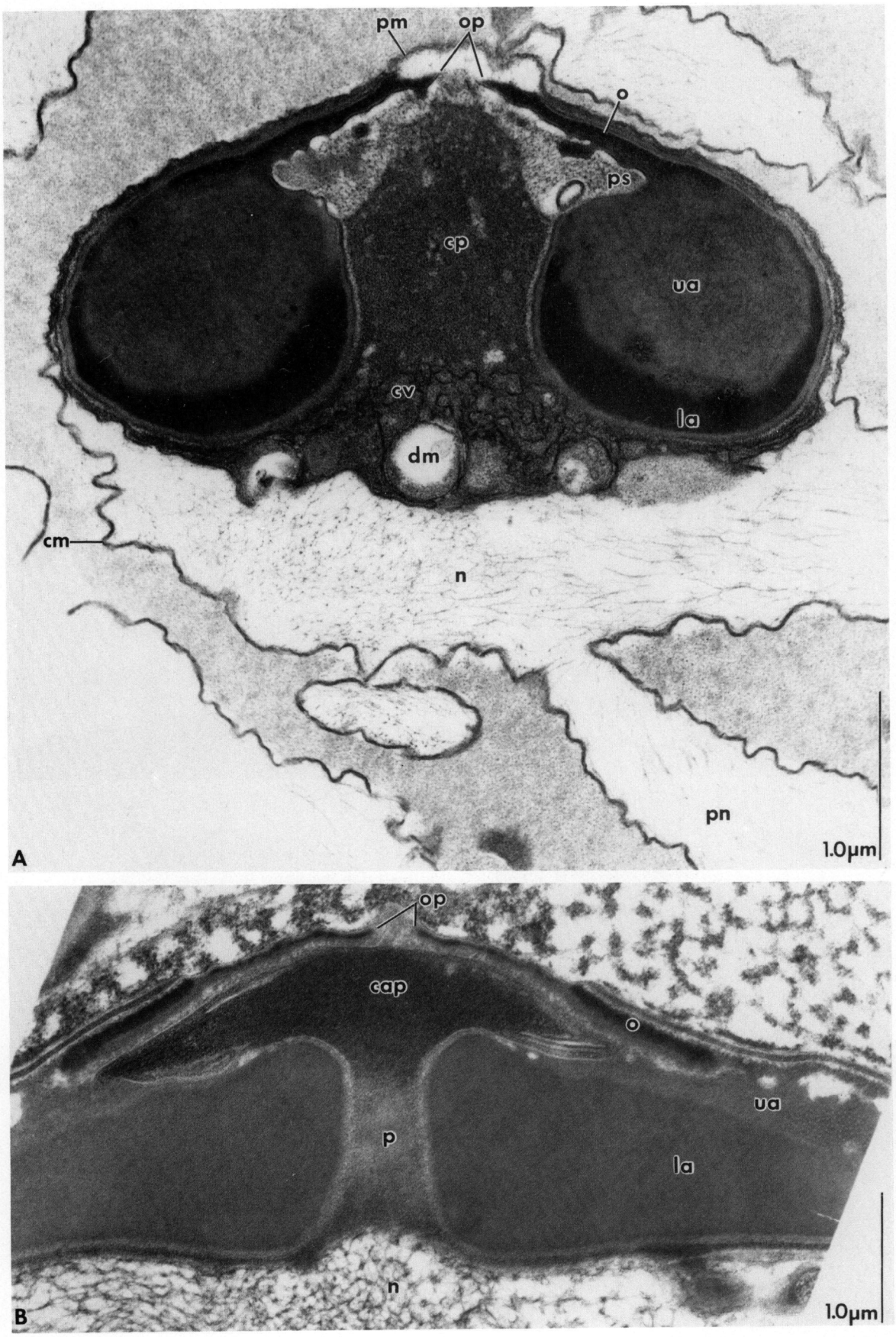

Fig. 5.—A. Transmission electron micrograph of a longitudinal sagittal section of a spermatozoon of *Homola* sp. for comparison with *B* a similar section through the perforatorium and adjacent regions of the sperm of the dromiid *Petalomera lateralis*.

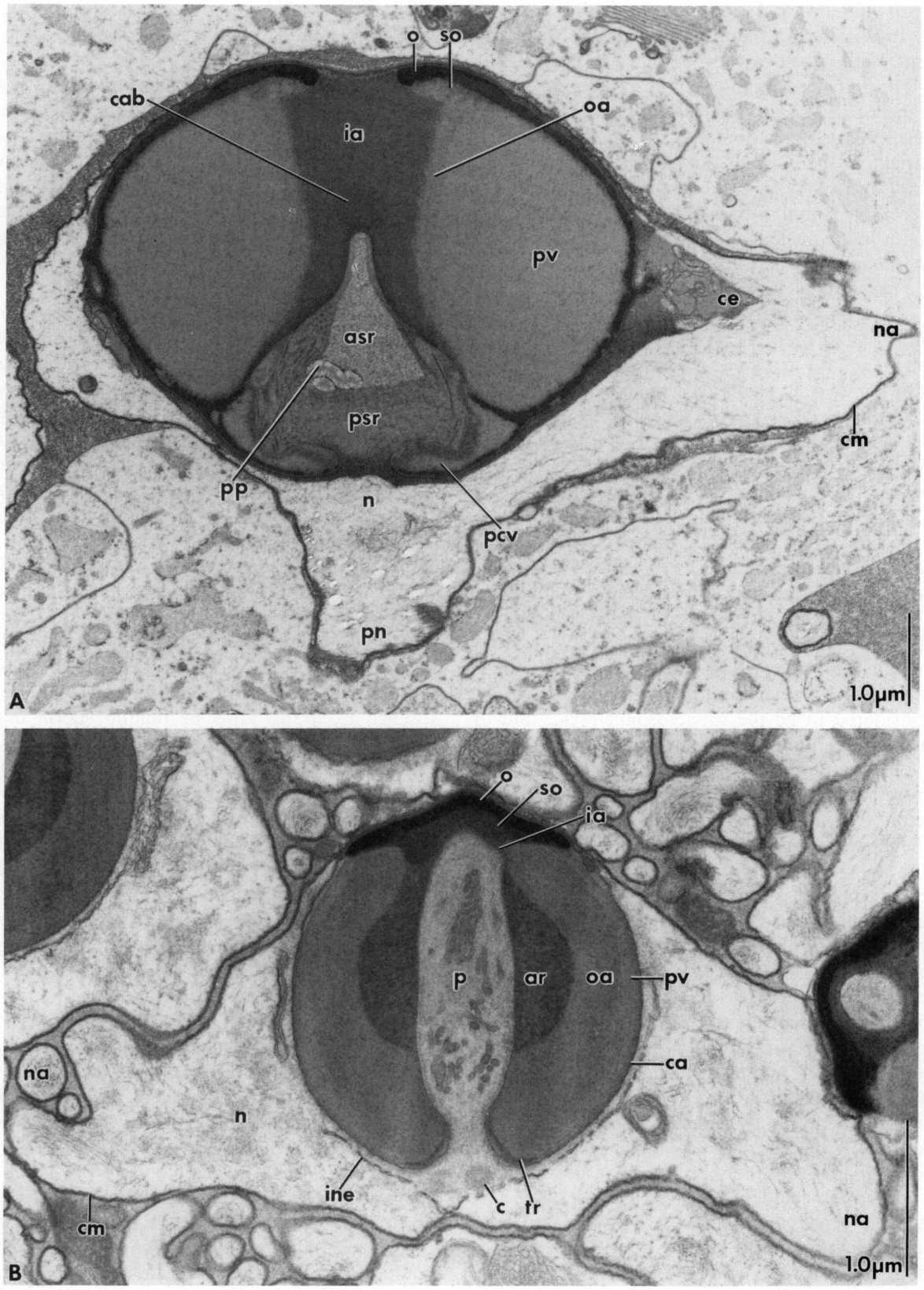

Fig. 6. Transmission electron micrograph of a sagittal longitudinal section of the spermatozoon of *A* the raninid *Ranina ranina* and *B* the portunid heterotreme *Portunus pelagicus*.

absence of a recognizable perforatorium and does not appear to be homologous with that in dromiids and homolids. Radiate structures more similar to, but again doubtfully homologous with, those of homolids are seen in the acrosome of the shrimp *Sicyonia ingentis* (see Kleve *et al.* 1980). In this shrimp there is a 'saucer-shaped plate' reminiscent of the head of the homolid capitate perforatorium. The unpaired anterior spike projecting at the tip of the shrimp acrosome vesicle is not seen in homolids.

Petalomera differs from homolids in that the acrosome is superficial on the nucleus, to which it presents an almost flat surface (see *Petalomera lateralis*, Jamieson 1990) whereas in homolids it is embedded approximately to its equator in the nucleus, though not as deeply embedded as in *Ranina* and heterotreme–thoracotreme crabs. However, in another dromiid, *Dromidiopsis edwardsi* Rathbun 1919, the acrosome is deeply embedded (Jamieson *et al.*, in prep.).

A broad area of the acrosome membrane at the anterior pole of the homolid acrosome is irregular (crenulated). In the dromiid *Petalomera* the central region of the operculum is also somewhat crenulate. In homolids, and as here shown to a lesser extent in *Petalomera* (and in *Dromidiopsis*) the operculum is interrupted centrally. In *Ranina* and Heterotremata the apical membrane is smooth; the operculum is apically interrupted in *Ranina* and in some Heterotremata.

Only in homolids does the perforatorium resemble the capitate perforatorium of dromiids in having a large anterolateral extension. In *Ranina* and Heterotremata–Thoracotremata the acrosome is penetrated by a broad central column. In *Ranina* the region of this considered to be the subacrosomal space and to be perforatorial is limited to a conical chamber which does not extend anterior of the equator of the acrosome. In Heterotremata the perforatorium forms a stout baton-like structure extending to the anterior end of the acrosome. The homolid perforatorium differs from that of dromiids in the spiked-wheel form of the anterior expansion, here interpreted as an autapomorphy for homolids.

Only in homolids does the acrosome, peripheral to the perforatorial chamber, resemble that of dromiids in being horizontally zonated (there is, however, both horizontal and concentric zonation in *Dromidiopsis*). In *Ranina* and Heterotremata–Thoracotremata the zonation is vertical and concentric. However, zonation in dromiids includes an acrosome ray zone not seen in homolids. The acrosomal rays also occur in the acrosomes of heterotremes, e.g. xanthids and portunids (Jamieson 1989*b*; Jamieson & Tudge 1990). Similar rays are, however, visible in published micrographs of the sperm of the astacids, *Pacifastacus leniusculus* (Dudenhausen & Talbot 1979) and *Cambarus* sp. (Anderson & Ellis 1967); and are well known in the sperm of hermit crabs (e.g. Hinsch 1980; Tudge 1992; Tudge & Jamieson 1991). They are therefore possibly plesiomorphic for reptantians.

There is no indication in homolids, dromiids or Heterotremata–Thoracotremata of the posterior subacrosomal region or of the posterior acrosomal chamber seen in *Ranina*.

Homolid sperm have irregular lateral arms but also (e.g. *Homolo* sp.), three radial nuclear vertices, little more than triangular projections, constituting short arms. Homolid arms contain only nuclear material as in *Ranina*, higher heterotremes and the Thoracotremata. Three 'stubby radial arms', lacking microtubular bundles, occur in *Dromidia antillensis* and apparently *Dromia vulgaris* (see Brown 1966; Grobben, 1878, respectively; both species junior synonyms of *Dromia personata* (Linné, 1758)) and are represented by three nuclear vertices in *Dromidiopsis edwardsi* (Jamieson *et al.*, in prep.). In *Petalomera lateralis*, although the ellipsoidal to subspherical nucleus frequently shows irregularities or distortions, no discrete arms were recognized ultrastructurally. Examination of further material of *Petalomera* is required, nevertheless, as it is possible that the three diminutive triangular prominences seen in *Dromidiopsis* are present. However, the plesiomorphic condition for heterotremes, seen in majiids, is the presence of arms which are nuclear but also contain bundles of microtubules. This is presumably the plesiomorphic condition for Heterotremata as it is also seen in other reptants, for instance, nephropids (see Talbot & Chanmanon 1980). Absence in brachyurans of purely microtubular arms is a notable distinction from anomurans such as the Paguroidea. Paguroid sperm otherwise have strong points of resemblance to heterotreme sperm which Jamieson (1993*b*) has considered indicative of relationship.

It is probable that absence of microtubules in the nuclear arms of dromiacean sperm is an independent loss representing a dromiid–homolid (and questionably raninid) synapomorphy. Absence of microtubules in the arms of higher heterotremes is clearly an independent and apomorphic loss from the majid-like condition. Absence from the arms of raninid sperm may be an independent development but could conceivably be synapomorphic with dromiids and homolids. We do not find evidence for a close raninid–dromiacean link.

Outside the Reptantia, arms questionably homologous with those of reptants have been reported for the sperm of the caridean shrimp *Rhynchocinetes typus* (Barros *et al.* 1986) and in branchiopods and Phyllocarida where they do not involve prolongation of the nuclear membrane, and are therefore probably not homologous with the thus characterized arms of decapods (see Jamieson 1991).

Similar in constitution to the nuclear arms is a posterior median process seen (transiently?) in homolid sperm, in *Ranina* and in majiids but absent from dromiid sperm. If homologous, this is, however, a symplesiomorphy as is seen in at least some paguroids (in some porcellanids it contains microtubules).

Presence of most of the cytoplasm (including tortuous membranes and degenerating mitochondria) below the acrosome is a homolid feature not seen in *Petalomera* (though seen in *Dromidia antillensis* with an intermediate condition in *Dromidiopsis*), nor in *Ranina* and the heterotreme–thoracotreme assemblage. In the absence of data on dynomenid and cyclodorippoid sperm it is difficult to establish that subacrosomal cytoplasm is a symplesiomorphy of dromiids and therefore of dromiids and homolids. In *Ranina* and the heterotreme–thoracotreme assemblage the small amount of cytoplasm is predominantly lateral to the acrosome with, in some heterotremes,

a trace posteriorly. In *Petalomera* there is the merest vestige of cytoplasm beneath the acrosome.

Centrioles have been observed in the cytoplasm posterior to the acrosome in homolid sperm. They are unknown in dromiids and raninids and are variable in occurrence in heterotremes. Their greatest development is seen in *Potamonautes* (Jamieson 1993*a*) and *Potamon* (Jamieson & Guinot, unpubl.), in the Heterotremata, in which they show a unique elongation. The presence of short centrioles is symplesiomorphic for brachyurans and is seen in many other decapods.

Phylogenetic and taxonomic implications

Similarities of homolid and *Petalomera* sperm noted above, especially the capitate perforatorium, the partly, at least, horizontal zonation of the acrosome vesicle, and the depressed form of the acrosome, support inclusion of the Dromiidae and Homolidae in a single grouping, the Podotremata. Until the sperm of dynomenids and cyclodorippoids are known, it will not, however, be possible to test the validity of the proposition of Guinot (1977, 1978, 1991), illustrated in Fig. 7, that homolids should be removed from the Dromiacea and placed, with cyclodorippids and raninoids, in the Archaeobrachyura.

In Fig. 7 the phylogeny of brachyurans suggested from non-spermatozoal characters by Guinot (1978, 1979) and by Guinot & Tavares (in prep.) is used as a framework for summarizing sperm structure in the investigated groups of crabs. Sperm ultrastructure has supported placing dromiids and homolids in the same clade but, in the absence of data on dynomenids and cyclodorippoids, does not contraindicate relegation of a homolid–cyclodorippoid subclade to the Archaeobrachyura, with or without the raninoids. The apparently apomorphic nature of the homolid perforatorium relative to dromiids suggests that homolids were a relatively late offshoot of the dromiacean stock.

It must be stressed, however, that there is very little in the ultrastructure of homolid or dromiid sperm to associate them with either the Raninidae or the heterotreme–thoracotreme assemblage. The major finding of the present study is the apparent close relationship of homolids and dromiids as evidenced particularly by the shared capitate perforatorium, by the horizontal zonation of the acrosome vesicle and, less cogently, the absence of microtubules in the arms, and the distinc-

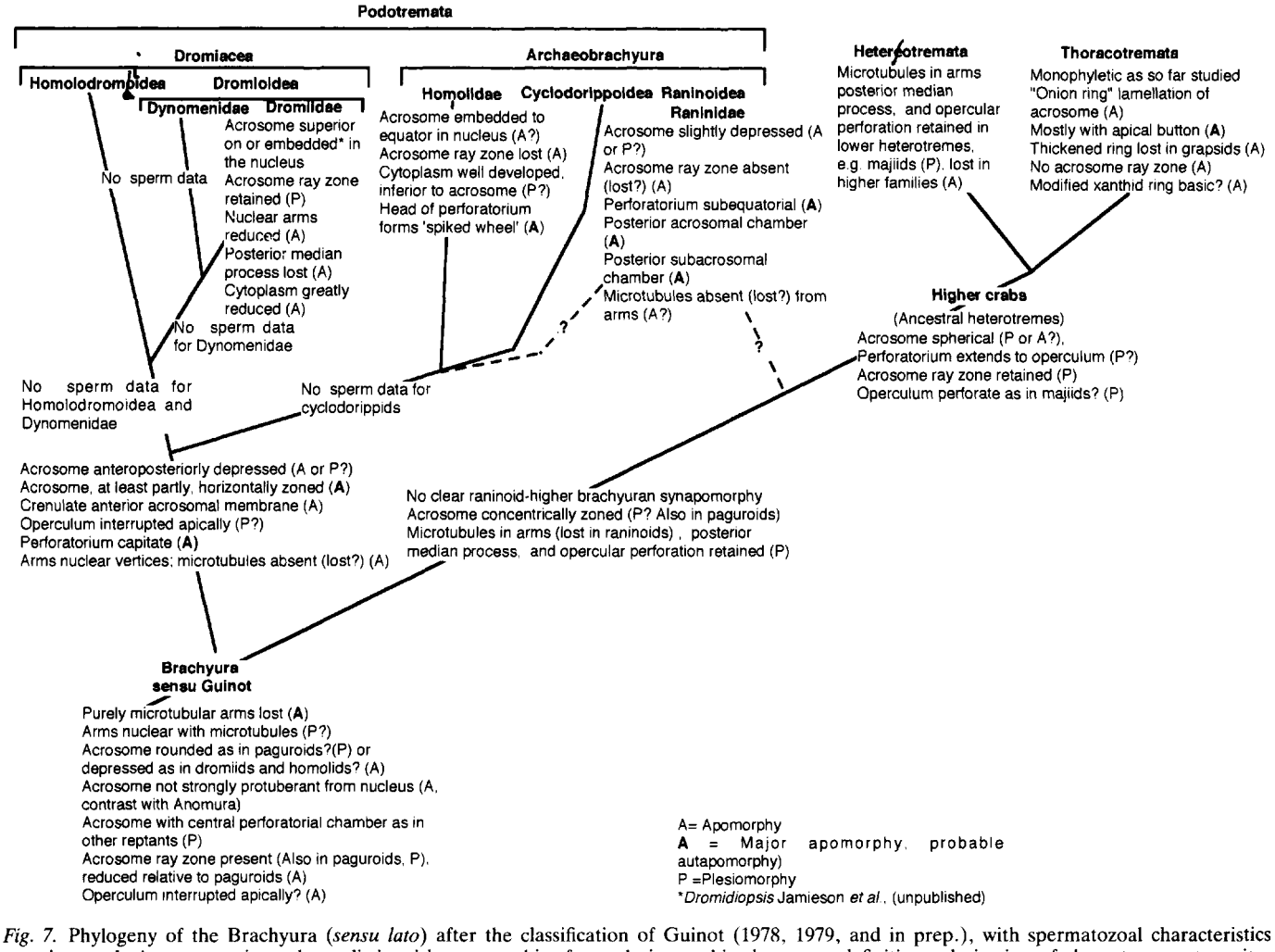

Fig. 7. Phylogeny of the Brachyura (*sensu lato*) after the classification of Guinot (1978, 1979, and in prep.), with spermatozoal characteristics superimposed. An attempt is made to distinguish apomorphies from plesiomorphies but more definitive polarization of characters must await a comprehensive review of anomuran and brachyuran spermatozoa. Note that if the Raninoidea are excluded from the Podotremata these and the Archaeobrachyura become paraphyletic groups.

tiveness of their sperm from those of raninids and heterotreme–thoracotremes.

The homogeneity of spermatozoal ultrastructure in the three species *Homola* sp., aff. *Paromola petterdi* and *Paromola* sp. provides few if any grounds for separating the three entities. Separation of these taxa as three distinct genera on the basis of somatic morphology has been argued by Guinot & Richer de Forges (in press). Spermatozoal homogeneity at the familial level, here the distinctive homolid type, is seen also in other crabs: dromiids (Brown 1966; Jamieson 1990), majiids (Hinsch 1973), xanthids (Jamieson 1989*b*), portunids (Jamieson 1991; Jamieson & Tudge 1990), and grapsids (Jamieson 1991), but species specific, if sometimes only metric, differences are observable and may yet prove to have taxonomic value. It remains to be seen whether small differences noted between homolid species, such as the more homogeneous composition of the perforatorium and the paracrystalline mitochondrial arrays in *Paromola* sp., or the lesser, though still strong depression of the acrosome in *Paromola petterdi* will prove to be reliable taxonomic characters for placement of these in distinct genera.

Acknowledgements

We are grateful to Mr C. Tudge for processing glutaraldehyde-preserved homolid material received from B. R. F. and for his careful reading of the manuscript. Mrs L. Daddow and Mr D. Scheltinga are thanked for excellent technical assistance. We thank Mr M. Tavares (Universidade Santa Ursula, Rio de Janeiro and Muséum national d'Histoire naturelle, Paris) for thoughtfully criticizing the earlier drafts of the manuscript and for his valuable comments. This work was made possible by Australian Research Council funding.

Abbreviations Used in the Figures

ar	acrosome ray zone
asr	anterior subacrosomal region
c	centriole
ca	capsule
cab	central acrosomal body
cap	capitate region of perforatorium
ce	cytoplasmic extension into arm
cm	cell membrane
cp	core of perforatorium
cv	convoluted membranes
cy	cytoplasm
dm	degenerating mitochondrion
eo	extensions of the operculum into head of perforatorium
ia	inner acrosomal zone
ine	disrupted inner nuclear envelope
la	lower acrosomal zone
n	nucleus
na	nuclear arm
o	operculum
oa	outer acrosomal zone
op	apical perforation of operculum
p	perforatorium
pac	paracrystalline material
pcv	posterior chamber of acrosomal vesicle
pm	plasma membrane
pn	posterior median process of nucleus
pp	putative perforatorium
ps	perforatorial spike
psr	posterior subacrosomal region
pv	peripheral contents of acrosome vesicle
so	subopercular zone
tr	thickened ring
ua	upper acrosomal zone

References

Abele, L. G. 1991. Comparison of morphological and molecular phylogeny of the Decapoda.—*Memoirs of the Queensland Museum* 31: 101–108.

Anderson, W. A. & Ellis, R. A. 1967. Cytodifferentiation of the crayfish spermatozoon: acrosome formation, transformation of mitochondria and development of microtubules.—*Zeitschrift für Zellforschung und Mikroskopische Anatomie* 77: 80–94.

Balss, H. 1957. Decapoda. VIII. Systematik. In H.G. Bronns (ed): *Klassen und Ordnungen des Tierreichs*, Funfter Band, 1. Abteilung 7, Buch 12, pp. 1505–1672. Winter, Leipzig & Heidelberg.

Brown, G. G. 1966. Ultrastructural studies on crustacean spermatozoa and fertilization. Ph.D. dissertation, University of Miami.

Burkenroad, M. D. 1963. The evolution of the Eucarida (Crustacea, Eumalacostraca), in relation to the fossil record.—*Tulane Studies in Geology and Paleontology* 2: 3–16.

Dudenhausen, E. & Talbot, P. 1979. Spermiogenesis in the crayfish, *Pacifastacus leniusculus*.—*Journal of Cell Biology* 83: 225a.

Glaessner, M. F. 1969. Decapoda. In R. C. Moore (ed.): *Treatise on invertebrate paleontology, Part R, Arthropoda*, 4, Vol. 2, pp. 399–532. The Geological Society of America, Boulder, Colorado.

Grant, F. E. 1905. Crustacea dredged off Port Jackson in deep-water.—*Proceedings of the Linnean Society of New South Wales* 30: 312–324, pls 10, 11.

Grobben, C. 1878. Beiträge zur Kentniss der männlichen Geschlechtsorgane der Dekapoden.—*Arbeiten aus den Zoologischen Instituten der Universität Wien und der Zoologische Station in Triest* 1: 57–150, pls 1–6.

Guinot, D. 1977. Propositions pour une nouvelle classification des Crustacés Décapodes Brachyoures.—*Compte Rendu Hebdomadaire des Séances de l'Académie des Sciences, Paris* D285: 1049–1052.

Guinot, D. 1978. Principes d'une classification évolutive des crustacés décapodes brachyoures.—*Bulletin Biologique de la France et de la Belgique* 112: 211–292.

Guinot, D. 1979. Données nouvelles sur la morphologie, la phylogenèse et la taxonomie des Crustacés Décapodes Brachyoures.—*Mémoires du Muséum national d'Histoire naturelle, Paris* (A) *Zool.* 112: 1–354, figs 1–70, pls 1–27, tables 1–5.

Guinot, D., 1991. Etablissement de la famille des Poupiniidae pour *Poupinia hirsuta* gen. nov., sp. nov. de Polynésie (Crustacea Decapoda Brachyura Homoloidea).—*Bulletin du Muséum national d'Histoire naturelle, Paris* (A) 12 (3–4) 1990 (1991): 577–606.

Guinot, D. & Richer de Forges, B. in press. Crustacea Decapoda Brachyura: Révisison de la famille des Homolidae de Haan, 1839.—*Mémoires du Muséum national d'Histoire naturelle, Paris* (A).

Gurney, R. 1942. Larvae of decapod Crustacea.—*Ray Society London* 129: 1–306.

Hinsch, G.W. 1973. Sperm structure of *Oxyrhyncha*.—*Canadian Journal of Zoology* 51: 421–426.

Hinsch, G. W. 1980. Spermiogenesis in *Coenobita clypeatus* I. Sperm structure.—*International Journal of Invertebrate Reproduction* 2: 189–198.

Jamieson, B. G. M. 1989*a*. Ultrastructural comparison of the spermatozoa of *Ranina ranina* (Oxystomata) and of *Portunus pelagicus* (Brachygnatha) (Crustacea, Brachyura).—*Zoomorphology* 109: 103–111.

Jamieson, B. G. M. 1989*b*. The ultrastructure of the spermatozoa of four species of xanthid crabs (Crustacea, Brachyura, Xanthidae).—*Journal of Submicroscopic Cytology and Pathology* 21: 579–586.

Jamieson, B. G. M. 1990. The ultrastructure of the spermatozoa of *Petalomera lateralis* (Gray) (Crustacea, Brachyura, Dromiacea) and its phylogenetic significance.—*International Journal of Reproduction and Development* 17: 185–189.

Jamieson, B. G. M. 1991. Ultrastructure and phylogeny of crustacean spermatozoa.—*Memoirs of the Queensland Museum* 31: 109–142.

Jamieson, B. G. M. 1993*a*. Ultrastructure of the spermatozoon of *Potamonautes perlatus sidneyi* (Heterotremata, Brachyura, Crustacea).—*South African Journal of Zoology* 28: 40–45.

Jamieson, B. G. M. 1993*b*. Spermatological evidence for the taxonomic status of *Trapezia* (Heterotremata, Brachyura, Crustacea).—*Memoirs of the Queensland Museum* 33: 225–234.

Jamieson, B. G. M. & Tudge, C. C. 1990. Dorippids are Heterotremata: evidence from ultrastructure of the spermatozoa of *Neodorippe astuta* (Dorippidae) and Portunus pelagicus (Portunidae) Brach-

yura: Decapoda.—*Marine Biology* 106: 347–354.
Kleve, M. G., Yudin, A. I. & Clark, W. H. 1980. Fine structure of the unistellate sperm of the shrimp, *Sicyonia ingentis* (Natantia).—*Tissue and Cell* 12: 29–45.
McKnight, C. E. & Hinsch, G. W. 1986. Sperm maturation and ultrastructure in *Scyllarus chacei.—Tissue and Cell* 18: 257–266.
Pike, R. B. & Williamson, D. I. 1960. Larvae of Decapod Crustacea of the families Dromiidae and Homolidae from the Bay of Naples.—*Pubblicazioni della Stazione Zoologica di Napoli* 31: 553–563.
Rice, A. L. 1970. Decapod crustacean larvae collected during the International Indian Ocean Expedition. Families Raninidae and Homolidae.—*Bulletin of the British Museum of Natural History* 21: 1–24, figs 1–9.
Rice, A. L. 1980. Crab zoeal morphology and its bearing on classification of the Brachyura.—*Transactions of the Zoological Society of London* 35: 271–424.
Rice, A. L. 1981*a*. Crab zoeae and brachyuran classification: a reappraisal.—*Bulletin of the British Museum (Natural History), Zoology* 40: 287–296.
Rice, A. L. 1981*b*. The megalopa stage in brachyuran crabs. The Podotremata Guinot.—*Journal of Natural History* 15: 1003–1011, fig. 1–3.
Rice, A. L. 1983. Zoeal evidence for brachyuran phylogeny. In F. R. Schram (ed.): *Crustacean phylogeny*. Crustaceans Issues, Vol. 1, pp. 313–329. Balkema, Rotterdam.
Rice, A. L. & Provenzano, A. J. 1970. The larval stages of *Homola barbata* (Fabricius) (Crustacea, Decapoda, Homolidae) reared in the laboratory. Biological Results of the University of Miami, Deep-sea expeditions, 55.—*Bulletin of Marine Science* 20: 446–471, figs 1–5, tables 1–3.
Spears, T. & Abele, L. G. 1988. Molecular phylogeny of brachyuran crustaceans based on 18S rRNA nucleotide sequences.—*American Zoologist* 28: 2A (Abstract).
Spears, T., Abele, L. G. & Kim, W. 1993. The monophyly of brachyuran crabs: a phylogenetic study based on 18S rRNA.—*Systematic Biology* 41: 446–461.
Stevcic, Z. 1971. The main features of brachyuran evolution.—*Systematic Zoology* 20: 331–340.
Talbot, P. & Chanmanon, P. 1980. The structure of sperm from the lobster, *Homarus americanus.—Journal of Ultrastructure Research* 70: 275–286.
Tudge, C. C. 1992. Comparative ultrastructure of hermit crab spermatozoa (Decapoda: Anomura: Paguroidea).—*Journal of Crustacean Biology* 12: 397–409.
Tudge, C. C. & Jamieson, B. G. M. 1991. Ultrastructure of the mature spermatozoon of the Coconut Crab *Birgus latro* (L.) (Coenobitidae, Paguroidea, Decapoda).—*Marine Biology* 108: 395–402.
Warner, G. F. 1977. *The biology of crabs*. Elek Science, London.
Williamson, D. I. 1965. Some larval stages of three Australian crabs belonging to the families Homolidae and Raninidae, and observations on the affinities of these families (Crustacea: Decapoda).—*Australian Journal of Marine and Freshwater Research* 16: 369–398.
Williamson, D. I. 1974. Larval characters and the origin of crabs (Crustacea, Decapoda, Brachyura).—*Thalassia Jugoslavica* 10: 401–414.
Williamson, D. I. 1988. Evolutionary trends in larval form.—*Zoological Symposium of the Zoological Society of London* 59: 11–25.
Williamson, D. I. 1992. *Larvae and evolution: toward a new zoology*. Chapman and Hall, London.
Wright, C. W. & Collins, J. S. H. 1972. British Cretaceous crabs. In *Paleontographical Society Monographs*, pp. 114. Paleontographical Society, London.